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14. ABSTRACT There is a high incidence of TBI among warfighter occupants of vehicles targeted by underbody blasts but little is known about the unique forces involved or the pathophysiology. We hypothesize: <ul style="list-style-type: none"> <li>Acceleration experienced during survivable underbody blasts produces dose-dependent, TBI.</li> <li>Underbody blast-induced acceleration combined with secondary head impact is also military relevant and can be modeled.</li> <li>Neurologic outcome following underbody blast-induced TBI can be improved by force-modifying vehicle hull designs.</li> <li>We will expand our underbody blast animal model of TBI to establish full dose-response relationships and to model the combination of acceleration plus head impact. This research will promote development of engineering- and biomedical-based neuroprotective interventions translatable to warfighter TBI.</li> </ul>					
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## INTRODUCTION:

There is a high incidence of TBI among warfighter occupants of vehicles targeted by underbody blasts but little is known about the unique forces involved or the pathophysiology. Our goal is to utilize small animal modeling of brain injury caused by underbody blasts to understand the pathophysiology of this uniquely military relevant form of TBI. Through this understanding, we aim to develop engineering- and biomedical-based neuroprotective interventions translatable to warfighter TBI.

Anesthetized and conscious animals are being used in experiments where the peak vertical acceleration elicited by an underbody blast will be varied between approximately 20 and 2000 Gs. Anesthetized rats will be used in additional experiments where the top of the head is allowed to strike the surface of the cylinder (cockpit), which models a combined insult typical of underbody blasts.

Comprehensive histopathology, multispectral magnetic resonance imaging, and behavioral tests are being performed at 2 hr to 30 days after these blasts to provide spatiotemporal quantification of diffuse axonal injury, vascular damage, cellular inflammatory responses, cell death, and neurologic outcome that are necessary for understanding and mitigating underbody blast TBI.

We have also made progress in demonstrating that survival can be improved by modification to vehicle hull designs, including the use of hull materials that reduce the rate and extent of blast-induced acceleration.

## BODY:

***ALL PROGRESS IS LISTED UNDER ORIGINAL SOW TECHNICAL REQUIREMENTS AND IS BOLDED AND ITALICISED***

### 1. Statement of Work

**1.0 Introduction:** The principal purpose of this agreement is to expand development of our novel, small animal model of TBI induced by the hyperacceleration associated with underbody blasts, with the long-term goal of supporting improved primary and secondary preventive strategies. All animal experiments and animal outcome measurements work will be carried out at University of Maryland School of Medicine. Experimental vehicle hull design and construction will be conducted at the University of Maryland School of Engineering.

#### 1.1 Summary of Specific Aims/Objectives:

1.1.1. Establish dose-dependent relationships between G-force/JERK, neuronal/axonal injury, neurochemical alterations, and inflammation in different brain regions at different times after the underbody blast in the absence and presence of secondary head impact.

1.1.2. Elucidate the neurobehavioral alterations that occur after underbody blasts and establish their temporal relationships with the nature and extent of neuropathology present in different brain regions.

1.1.3. Determine if alterations in vehicle hull design, particularly those that reduce both maximal G-force and JERK, reduce histologic, neurochemical, or behavioral indices of brain injury.

#### 2.0 Technical Requirements:

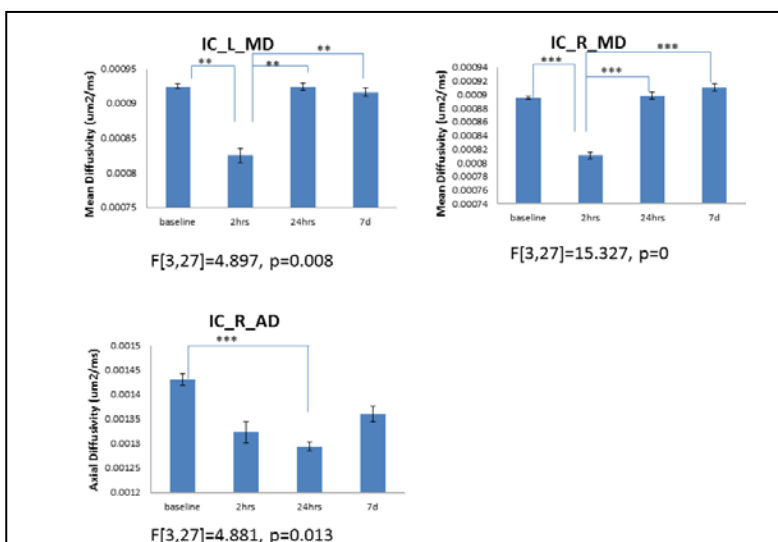
**2.1.** Quantify physiologic, neurochemical, and neuro-histopathologic TBI outcomes after exposure of rats to underbody blast-induced hyperacceleration. Compare these outcomes to direct measurements of acceleration (G-force) and acceleration rate (JERK) to determine minimal and maximal survivable loads associated with TBI, to establish dose-dependence relationships, and to identify the neurobiologic alterations most closely linked with the pathophysiology of this form of TBI. (Aligned with Objective 1)

2.1.1. Expose anesthetized test animals (rats) to defined degrees of blast-associated acceleration forces while secured on a metal structure that simulates a closed armored vehicle.

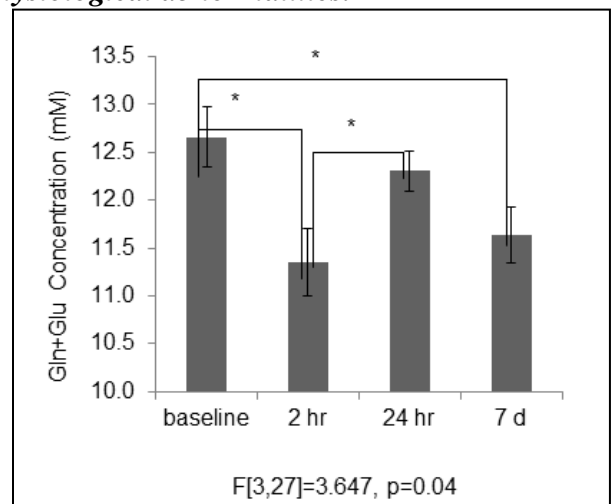
*Approximately 65 ketamine-anesthetized rats have been subjected to underbody blasts resulting in peak vertical accelerations ranging from 100 G to 2800 G. All rats survived blasts ranging from 100 to 2000 Gs. Five of six rats exposed to 2800 G died immediately or within 30 min post-blast. .*

2.1.2 Utilize a subset of animals for MRI and MRS measurements performed at one day prior to blast exposure (baseline) and again at several times post-blast.

*Eight of the rats exposed to 2000 G underbody blast were used for MRI/MRS measurements performed at baseline (one day prior to blast), and 2 hr, 24 hr and 7 days post-blast. Representative preliminary results from diffusion tensor imaging (DTI) and magnetic resonance spectroscopy (MRS) are shown in Figures 1 and 2. Mean diffusivity of water is reduced at 2 hr post-blast and returns to normal at 24 hr and 7 days. Axial diffusivity appears reduced at 2 hr, 24 hr, and 7 days post-blast but is only significantly lower with n=6 animals at 24 hr. These changes could represent intraxonal molecular alterations which are consistent with the silver staining observed in the internal capsule of animals subjected to underbody blasts. MRS measurements of glutamate plus glutamine indicate a significant reduction in these metabolites in the cerebral cortex at both 2 hr and 7 days post-blast. These changes could represent metabolic alterations in either neurons or astrocytes since most glutamate is present in neurons and most glutamine is in astrocytes. Preliminary DTI and MRS measurements have also been performed in the hippocampus (not shown). The significance of these results is that they provide evidence from non-invasive measurements that exposure of rats to underbody blasts results in both neurochemical and physiological abnormalities.*



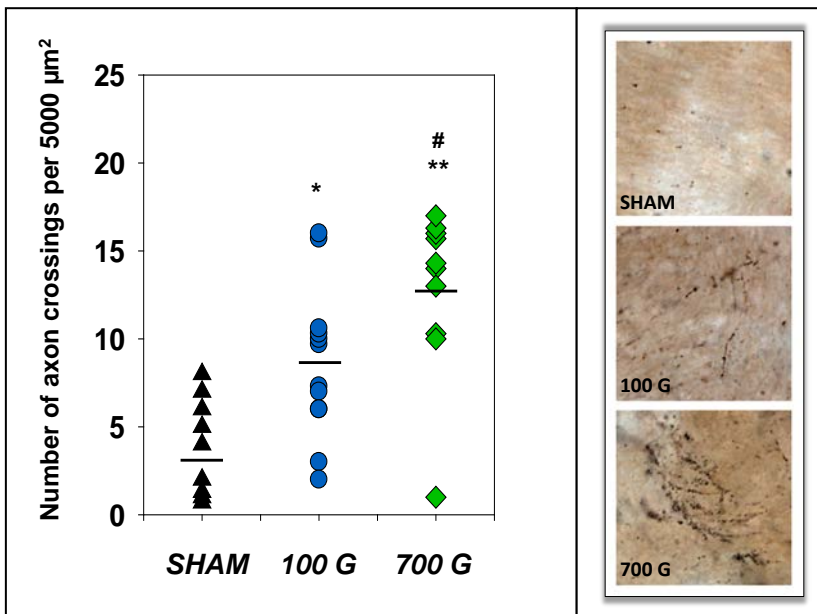
**Fig. 1. Diffusion tensor imaging of water diffusion in the internal capsule before and after 2000 G underbody blast.** Mean diffusivity changes in the left (L) and right (R) are bisymmetric and observed primarily at 2 hr post-blast. Axial diffusivity is also reduced after blasts. N=6 animals.



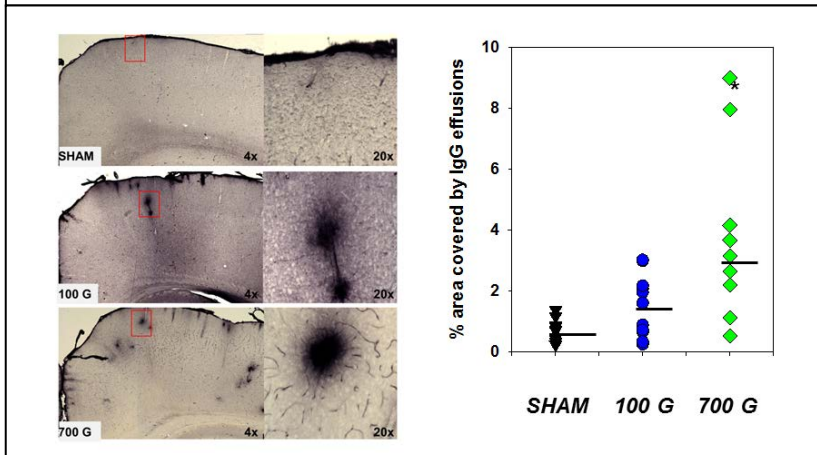
**Fig. 2. Magnetic resonance imaging of total glutamine plus glutamate present in the frontal cortex before and after 2000 G underbody blast.** Significant reductions were observed at both 2 hr and 7 days post-blast. N=6 animals.

2.1.3. Euthanize anesthetized rats by perfusion fixation within 2 hr after blast exposure, remove brains, and process for electron microscopic analysis of cyto- and axonal ultrastructure and for histochemical evidence of acute neurochemical and neuroanatomic alterations.

*Four ketamine-anesthetized rats exposed to 700G blast were perfusion fixed at 20 min post-blast and their brains processed for electron microscopy. We obtained quantitative evidence for perivascular swelling of astrocyte end-feet and swelling of vascular endothelial cells.*



**Fig. 3.** Silver staining of damaged axonal fibers present in the internal capsule at 7 days after 100 or 700 G blasts or sham anesthesia. \*p<0.05 compared to sham. #p<0.05 compared to 100 G. N=8-11 animals per group.



**Fig. 4.** Immunoglobulin G immunostaining in the cerebral cortex of rats at 7 days after 100 or 700 G underbody blasts. Percent area covered by perivascular IgG effusions is significantly greater after 700 G blast compared to sham anesthesia controls (\*p<0.05). N=7-9 animals per group.

2.1.4 Euthanize anesthetized rats by perfusion fixation at < 2hr and 24 hr, and at 7 and 30 days post-blast, and processed brains for quantitative histochemical and biochemical evidence of subacute and chronic neurochemical and neuroanatomic alterations.

*Animals have been perfusion fixed at 2 and 24 hr post-blast and at 7 and 30 days post-blast. At this juncture, most of our quantitative histopathology was generated from animals at 7 days post-blast. As shown in Fig. 3, there was a significant increase in silver staining (de Olmos method) of axon fibers present in the internal capsule of animals exposed to 100 and 700 G underbody blasts compared to ketamine-anesthetized shams. There was also a significant difference between axonal injury in the internal capsule of rats subjected to 700 Gs compared to 100 Gs or to shams. Immunohistochemistry for immunoglobulin G was used as a measure of blood-brain barrier (BBB) disruption since normally IgG is present at only very low levels within the brain parenchyma of sham animals with an intact BBB. Fig. 4 provides the total area of perivascular IgG immunostaining in the frontal cerebral cortex. No significant increase was observed in animals following 100 G underbody blasts; however, there was a significant increase in perivascular IgG effusion area following 700 G blasts.*

*Additional qualitative findings were obtained at the lower end of the G force range and are included in our published*

*manuscript (Proctor et al (2014), Journal of Trauma and Acute Care Surgery) (see Appendix). These observations include increased axonopathy (silver staining) in the cerebellum and astrocyte activation in the cerebral cortex. The significance of these quantitative histologic measurements is that they strongly suggest that exposure of rats to survivable underbody blasts results in both white matter axonopathy and vascular injury resulting in disruption of the blood brain barrier.*

*We have also initiated our neurochemical analyses using RNA extracted from hippocampi within unfixed brains. Rats were euthanized, their brains quickly removed, and dissected into regions containing the hippocampus, cortex, internal capsule, and cerebellum, which were stored under -80°C. Total RNA was extracted with TRIzol. The quality and quantity of extracted RNA was further analyzed using Agilent 2100 Bioanalyzer. RNA with integrity number above 7 was used for microarray analysis. All procedures were performed with DNase- and RNase-free tools and reagents.*

*RNA was amplified and biotin labeled using the two-cycle target labeling kit (Affymetrix) and hybridized to the GeneChip® Rat Gene 2.0 ST arrays (Affymetrix). Samples were processed by the Biopolymer-Genomics Core Facility at the University of Maryland Baltimore. Samples were hybridized using a GeneChip hybridization oven 640 (Affymetrix), processed using a GeneChip Fluid Station 450 (Affymetrix), and scanned using a GeneChip Scanner system 3000 7G (Affymetrix). Microarray data normalization was performed with Affymetrix® Console™ software.*

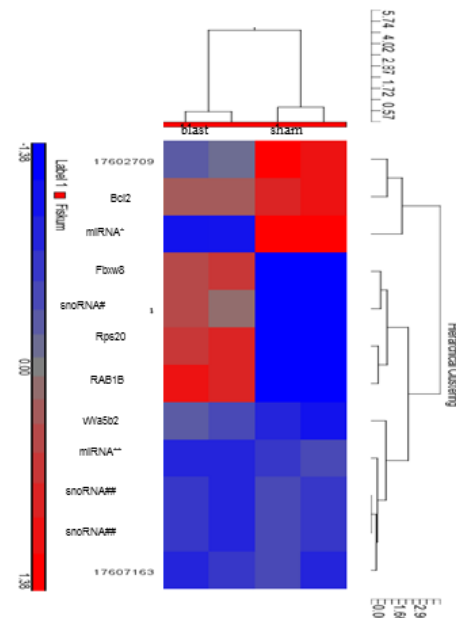
*The advantage of using GeneChip® Rat Gene 2.0 ST array over the other related products is the high transcript coverage (every exon of every transcript is probed, and median of 22 probes per gene) that yields accurate detection for genome-wide transcript expression changes. Furthermore, more transcripts are covered (> 27,000 protein coding transcripts, >23,500 Entrez genes) that allows novel target discoveries.*

*CEL data files were analyzed using Partek Genomics Suite version 6.4 (Partek GS, Partek, Inc.). Data were subjected to filtering by the detection of p-value and Z-normalization. Genes were identified as differentially expressed after calculating the Z-ratio, which indicates the fold-difference between experimental groups, as well as false discovery rate (FDR), which controls for the expected proportion of false rejected hypotheses. ANOVA was performed to evaluate the significance in gene expression alteration between experimental groups. Of the over 7500 genes identified as changing in expression in the hippocampus at 24 hr after 100 G underbody blast, 12 genes were identified as changing in expression very significantly, using rigorous criteria of  $p \text{ value} \leq 0.05$ , absolute value of  $z\text{-ratio} \geq 2.0$  and  $FDR \leq 0.05$ . These genes are listed in Fig. 5, along with a heat map demonstrating changes in expression between blast animals and shams and the finding that there was excellent agreement between the two animals in each group. Several microRNAs and small nuclear RNAs were identified, which likely play roles in regulating gene expression. Exposure to the blast resulted in a large decrease in Bcl2 expression, which could render brain cells highly vulnerable to apoptotic death. Blast exposure also induced a large increase in expression of the gene coding for VonWillebrand factor, which is known to increase during adverse changes to cerebral endothelial cells and can increase risk for thrombosis. This finding was recently validated by qPCR, demonstrating a 10 fold increase in von Willebrand factor mRNA levels in the hippocampus at 24 hr after the 100G blast (Fig. 6).*

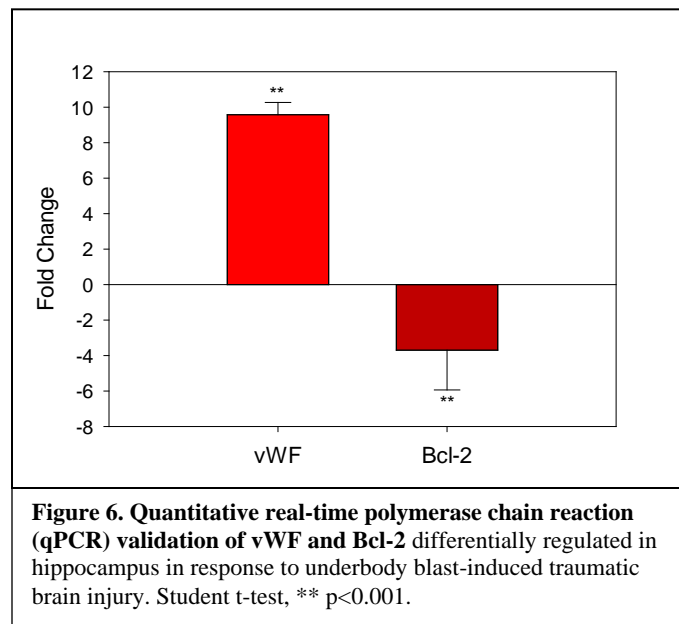
*Other gene expression cluster analyses are in progress to determine if changes occur in sets of genes, e.g. those associated with inflammation, oxidative stress, etc. The significance of these results is that significant changes in gene expression occur in the rat hippocampus within 24 hr following even the relatively low level 100 G underbody blast. Studies are in progress to determine gene expression changes following both higher and lower G force blasts.*

Fold change  $\geq 2$ ,  $p \leq 0.05$ ,  $FDR \leq 0.05$

Gene Symbol	RefSeq	p-value	Fold-Change	Description (100G vs. sham)
Novel miRNA*	ENSRNOT0000043523	1.01E-05	-2.06438	down
Bcl2	ENSRNOT00000003768	4.07E-05	-2.59557	down
Fbxw8	reviewed	8.99E-05	2.86312	up
Novel miRNA**	ENSRNOT000000063683	0.000312	2.13415	up
RAB1B	ENSRNOT00000027486	0.000617	2.47881	up
Rps20	NM_001007603	0.000589	2.50095	up
snoRNA##	ENSRNOT000000069073	0.000631	2.00346	up
snoRNA##	ENSRNOT000000069073	0.000631	2.00346	up
-	---	0.000991	2.83156	up
snoRNA#	ENSRNOT000000054102	0.000652	2.28636	up
-	---	0.000988	-2.94326	down
Vwa5b2	NM_001134535	0.001112	2.22691	up



**Fig. 5. Major gene expression changes in the hippocampus at 24 hr following exposure to 100 G underbody blast, compared to sham controls.** The heat map demonstrates relative differences between blast (left) and sham (right) and also the similarities between the two animals in each group.



**Figure 6. Quantitative real-time polymerase chain reaction (qPCR) validation of vWF and Bcl-2 differentially regulated in hippocampus in response to underbody blast-induced traumatic brain injury.** Student t-test, \*\*  $p < 0.001$ .

2.1.5. Compare quantitative histopathologic measurements of brain injury with accelerometer measurements of maximal G-force and JERK.

*At this juncture, most of our histopathologic measurements have been performed on the brains of rats exposed to 100, 700, and 2000 G and perfusion fixed at 7 days post-blast. Additional experiments are in progress to establish quantitative relationships between both G-force or JERK and histopathologic outcome measures. Specifically, we can achieve the same peak G force but a substantially greater JERK (dG/dt) by manipulating both the weight of the simulated vehicle, the size of the explosive, and the explosive stand-off distance.*

2.1.6. Milestone: Complete all histopathologic marker/G-force correlations (*timing = 24 months*).

*While we have made considerable progress using histopathology to understand the pathophysiology of underbody blast-induced TBI, many more experiments and histologic tests are necessary. Taken together with the new experiments that will combine blast-induced acceleration with head impact, the histopathology measurements will likely continue during the duration of the project.*

2.1.6.1. Deliverable 1: Determination of whether histopathologic markers of brain injury display a dose-dependent relationship to underbody blast-associated G-forces and whether maximal Gs, JERK or HIC is the best predictor of TBI.

2.1.4.2. Deliverable 2: Determination of minimum G-force associated with any degree of measurable neurohistopathology; determination of the maximum survivable G-force in this experimental system.

*At this juncture, for ketamine anesthetized rats, the minimum acceleration that yields quantifiable histopathological evidence for brain injury is 100G. The maximum survivable acceleration associated with both histologic and neurologic evidence for TBI is 2000G, for ketamine-anesthetized rats. We know that conscious rats can survive 2800G but will soon submit an addendum to our IACUC and then the ACURO to expose wide-awake rats to 4000G blasts.*

## **2.2. Quantify neurobehavioral alterations after underbody blast-associated acceleration injury, including any evidence of G-force and JERK dose-dependence. (Aligned with Objective 2)**

2.2.1. Apply these tests to experiments described by 2.1.1.

2.2.2. In animals surviving to 30 days post-injury, perform behavioral testing with standard methods

2.2.3. Correlate results of neurobehavioral testing with measured G-force and JERK.

2.2.4. Compare results of neurobehavioral testing with MRI/MRS and histologic measurements.

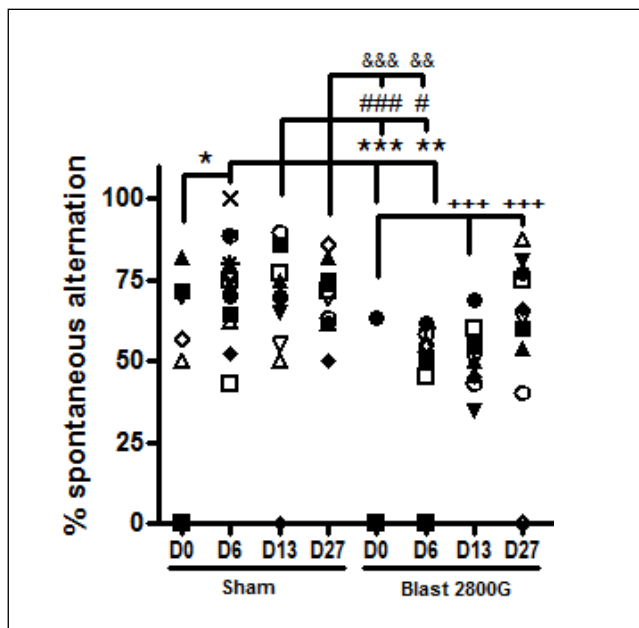
2.2.5. Milestone: Complete all neurobehavioral testing/G-force correlations (*timing = 24 months*).

*Initial neurobehavioral test included the balance beam, testing for both latency crossing the beam and the number of foot faults. In addition, a Composite Neuroscore was used for a rough assessment of neurological status. An Open Field test was also used, where distance traveled, time immobile, time in inner zone, and time in outer zone were recorded. Finally, we have recently included the forced swim tests, as a measure of depressive behavior. At this juncture, we have generated preliminary results for animals that were exposed to 700 G underbody blasts (8) compared to the ketamine anesthetized shams (5). In general, no differences have been observed, except that significantly more foot faults were observed with 700 G blast rats measured at 14 days compared to shams.*

*We then performed the same tests on wide-awake animals exposed to 700G blasts. In addition to the tests performed from 1 to 28 days post-blast that were performed with anesthetized rats, we performed composite neuroscore and beam walking tests on the conscious rats within 30 min of the blasts. These rats exposed to 700G blasts took longer to cross the beam and exhibited a lower neuroscore than the Sham rats that underwent the brief isoflurane anesthesia but were not exposed to the acceleration. During this time we started validation of two new tests, the Plus Maze and the Y Maze. Naïve rats exhibited normal behavioral characteristics for each of these tests. Specifically, they alternated exploration of each of the 3 closed arms of the Y maze and spent most of their time in the two open arms rather than the two closed arms of the Plus maze. We therefore applied these tests to the conscious animals exposed to 2800G blasts, hypothesizing that these tests would be more sensitive indicators of mild TBI than the previous tests that are normally applied to moderate or severe TBI rodent models.*



As shown in Fig. 7, spontaneous alternation between the 3 corridors of the Y maze was significantly reduced at both 1 hr and 6 days following exposure of conscious rats to 2800G blast. The animals exposed to blast improved significantly between 6 and 27 days post-blast.



**Fig. 7. Y maze test indicating reduced exploratory behavior following exposure to 2800G blast.** Sham animals at days 6, 13 and 27 post-injury had a significantly higher % spontaneous alternation of the Y maze arms than blast-injured (2800G) animals 1 hr and 6 days post-injury. Statistical significance comparing sham animals to blast animals at different study days is indicated in the graph by \*\*\* $p < 0.001$ , and \*\* $p < 0.01$  (to compare Sham at D6 –Pi to blast animals at days 1 and 6 pi respectively), ### $p < 0.001$ , and # $p < 0.05$  (to compare Sham at D13 –Pi to Blast animals at days 1 and 6 pi respectively) and &&& $p < 0.001$ , and && $p < 0.001$  (to compare Sham at D27 –Pi to Blast animals at days 1 and 6 pi respectively). In addition, blast animals showed significant recovery from the blast induced impairment in working memory at days 13 and 27 post-injury compared to their status an hour after injury (+++ $p < 0.001$ ). Statistics: Analysis of variance with Tukey-Kramer Multiple Comparisons Test

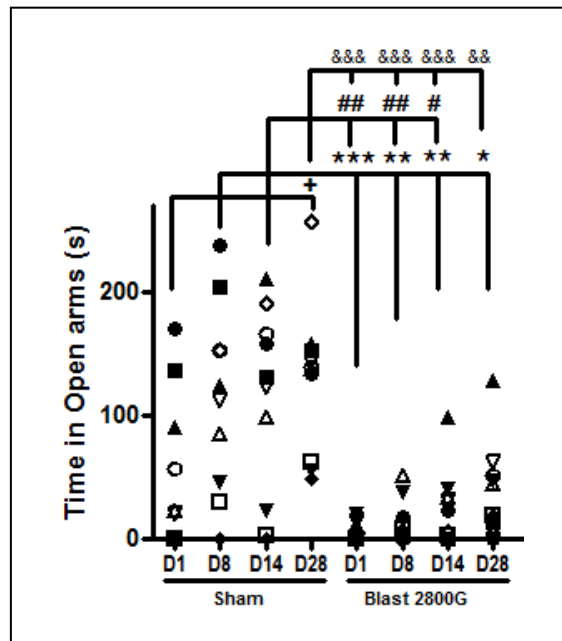


Fig. 8. Elevated plus maze indicating reluctance to spend time in the open, which is a measure of fear or anxiety. Animals exposed to 2800G blast spend significantly less time in the open arms of the maze at up to 14 days after blast.

As shown in Fig. 10, the time the blast-exposed, conscious rats spent in the “open” arms that were exposed to the surrounding environment was significantly shorter than the time spent in the open by Sham animals at up to 28 days post-blast. These results are very important as they represent the first indication for prolonged neurobehavioral deficits caused by exposure to underbody blasts.

2.2.5.1. Deliverable: Determination of whether neurobehavioral testing results display a dose-dependent relationship to blast-associated G-force or JERK.

2.2.5.2 Deliverable: Identification of the physiologic, neuroanatomic, and neurochemical outcome measures that are most closely related to neurobehavioral indicators of TBI, thus providing insight into the pathophysiology of this form of TBI.

### 2.3 Perform a limited number of experiments and outcome measures described in 2.1 and 2.2 with rats that are fully-awake but restrained during the underbody blast. (Aligned with Objectives 1 and 2)

2.3.1. In addition to standard outcome measurements, determine if rats lose consciousness or ability to walk or right themselves after moderate G force underbody blasts.

2.3.2. Quantitatively compare both short and long-term outcome measurements obtained from rats that are anesthetized and those that are conscious.

2.3.3. Milestone: Complete all blasts and outcome measurements with rats wide-awake during the blasts (timing = 30 months).

*Approval was obtained from the Univ. of Maryland, Baltimore IACUC and the ACURO to expose conscious rats to blasts. We then purchased standard plexiglass rat restraints from Braintree Scientific and bolted two of these to the top hull of our blast device, in place of the aluminum tubes used previously for the ketamine-anesthetized rats. The rats fit tight within these restrains allowing for normal breathing but very little movement of the head or body that could cause external or internal injuries. To eliminate the stress of placing the rats into the restrains, we exposed them to 5% isoflurane for 5 min and then secured them in the restrains while they were briefly anesthetized. Within 2 min of removing them from the isoflurane chamber, the rats began to move. They appeared completely awake 5 min after cessation of anesthesia, which is when*

*the explosive was detonated. The rats were then immediately tested, using the Plus and Y Mazes. The results are shown above composite neuroscore and beam walking tests. Histopathology on the brains of these animals is in progress.*

2.3.3.1. Deliverable: Determination of whether rats that are conscious during exposure to underbody blasts demonstrate immediate neurobehavioral alterations and if they exhibit evidence for greater short-term and long-term TBI compared to animals anesthetized during the blast.

**2.4. Establish a modified version of the animal model that includes a controlled secondary head impact during the underbody blast-induced hyperacceleration. (Aligned with Objectives 1 and 2)**

2.4.1. Develop an articulated rat head holder that allows for the top of the skull to impact the “roof” of the vehicle during the underbody blast.

2.4.2. Expose *anesthetized* rats to a moderate G force underbody blast, allowing for secondary head impact.

2.4.3. Perform all outcome measurements described in 2.1 and 2.2.

2.4.4. Quantitatively compare outcomes obtained from rats with secondary head impact to those without secondary head impact.

2.4.5. Milestone: Complete all blast experiments and outcome measurements with rats exposed to underbody blast plus secondary head impact (*timing = 36 months*).

*We anticipate initiation of these experiments within the next 6 months.*

2.4.5.1. Deliverable: A small animal model of TBI caused by the combination of underbody blast-induced hyperacceleration plus secondary head impact that is particularly relevant to many of the warfighters that survive underbody blasts.

**2.5. Test the effects of different vehicle hull designs on the loads imparted to the vehicle and to the test animals and determine which design is most effective at reducing TBI. (Aligned with Objective 3)**

2.5.1. Test 3 different hull designs (e.g., multiple V-hull and inverted V-hull) for mitigation of maximal G force and JERK loads on the vehicle alone.

2.5.2. Test 3 of these design modifications with *anesthetized* rat occupants at a blast stand-off distance that imparts a moderate G-force with the standard hull design.

2.5.3. Perform all outcome measurements described in 2.1 and 2.2.

2.4.4. Quantitatively compare outcomes obtained from tests using the modified hull designs to those using the standard hull design.

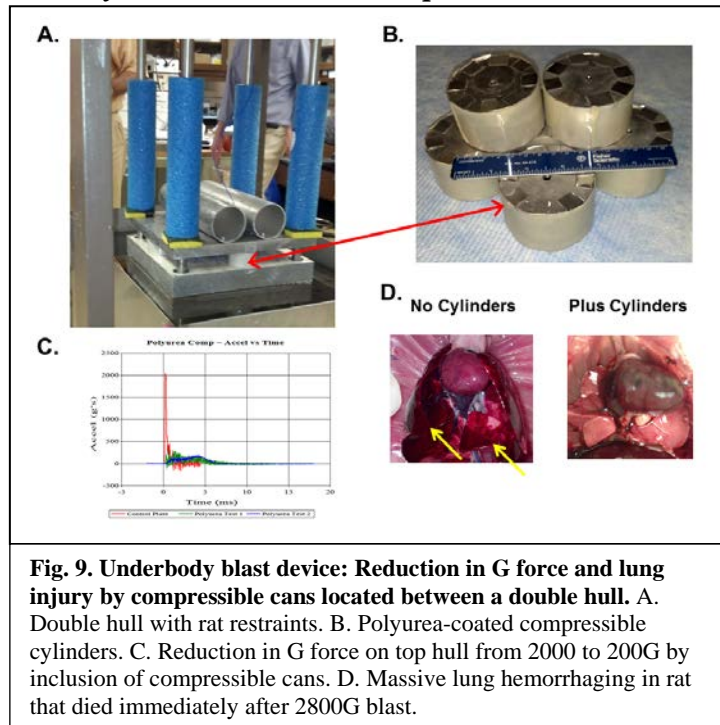
2.4.5. Milestone: Complete all blast experiments and outcome measurements with the modified hull designs (*timing = 44 months*).

*Substantial progress has been made toward this objective, as shown below.*

2.4.5.1. Deliverable: Identification of a hull design that both mitigates loads on the vehicle and its occupants and that reduces TBI after underbody blast.

## 1. Improving survival from underbody blasts by a double hull separated by compressible cylinders

*Dr. Fourney's Dynamic Effects Laboratory at the University of Maryland College Park School of Engineering has for many years been using small scale simulated vehicle models and subjected them to a variety of blast conditions <http://www.cecd.umd.edu/projects/dynamic-effects-lab.html>. He has tested a wide variety of vehicle hull designs for their ability to withstand blasts. His work has been validated with full scale vehicles and helped lead to the double V-hull design used in "mine resistant, ambush protected military vehicles (MRAPs) that have dramatically reduced the number of mortalities associated with vehicles targeted by IEDs. As an integral part of this project, Dr. Fourney and his associates, Dr. Leiste and Dr. Bonsmann, have tested the ability of compressible cylinders located between top and bottom hulls to reduce the load and therefore the acceleration of the top hull, i.e., farthest away from an underbelly blast. As mentioned in a previous progress report, they have found that the placement of compressible polyurea-coated aluminum cylinders between two hulls can reduce the load imparted to the top hull by approximately 90%, compared to the acceleration measured when only a thin rubber mat was present between the two hulls. Given this result,*



**Fig. 9. Underbody blast device: Reduction in G force and lung injury by compressible cans located between a double hull. A. Double hull with rat restraints. B. Polyurea-coated compressible cylinders. C. Reduction in G force on top hull from 2000 to 200G by inclusion of compressible cans. D. Massive lung hemorrhaging in rat that died immediately after 2800G blast.**

*we tested the compressible can design in our underbody blast model with ketamine-anesthetized rats secured within restraints bolted to the top of the upper hull (Fig. 9.A).*

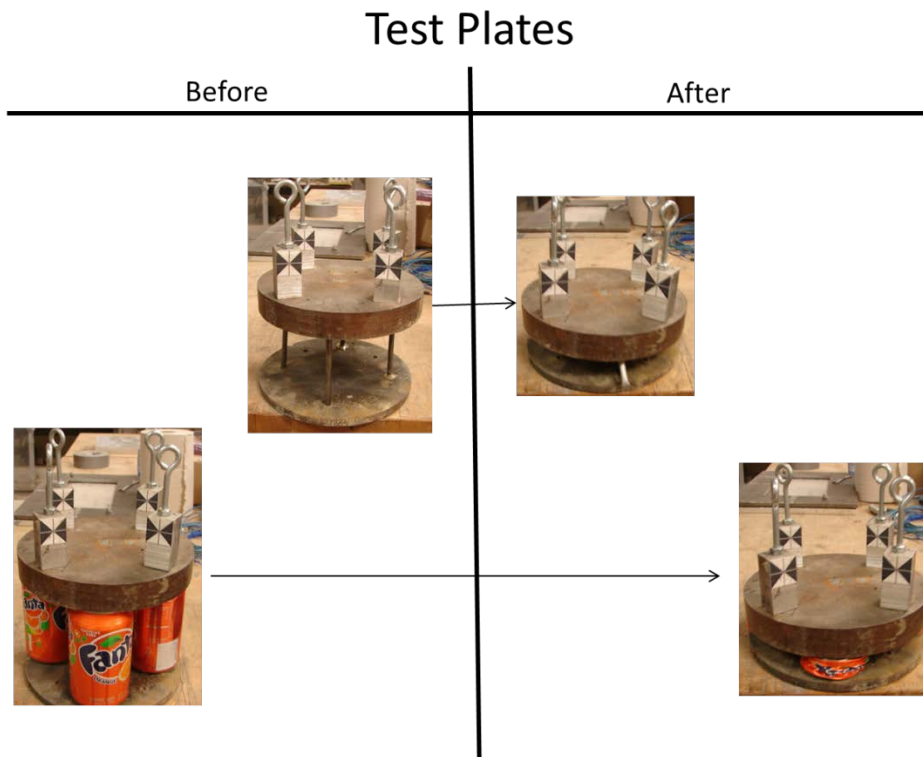
*For these tests, we used a relatively large, 2.3 g level of pentaerythritol tetranitrate as the explosive and a stand-off distance between the charge and the bottom of the lower plate that would give a peak acceleration of the top hull of 2800G in the absence of the compressible cylinders. As shown in Fig. 9.B, a maximum force of 2800G was indeed obtained, using an accelerometer located directly next to the head end of tubes in which the ketamine anesthetized rats were placed. In the presence of 4 compressible cylinders located just inside the edge of the hulls halfway between each corner plus one located between the centers of each hull, the maximum acceleration measured on the top hull was reduced to between 150 and 300G. Using two rats for each blast, 5 out of 6 that were subjected to the 2800G force died either immediately or within 30 min later due to massive internal hemorrhaging to the lungs, liver, and other organs (Fig. 9.D). In contrast, all 8 rats survived that were subjected to the same conditions except that the cylinders were present between the top and bottom hulls. These rats appeared healthy and gained normal weight during the 7 days they were kept alive before perfusion fixation. At the time of fixation, all internal organs appeared normal (Fig. 9.D). We consider these results to be highly significant as they represent strong evidence that a relatively simple modification of a vehicle hull design can dramatically reduce the load on occupants during an underbody blast, thereby saving lives. As previously communicated, Dr. Fourney submitted a hull design patent application based on results he obtained in his Dynamic Effects laboratory. Hopefully, he can partner with*

*the US Army and or military contractors to translate this design into a new and much safer class of MRAPs. The following provides additional details regarding the previous and ongoing tests performed at the Dynamic Effects Laboratory that are supported by this research award.*

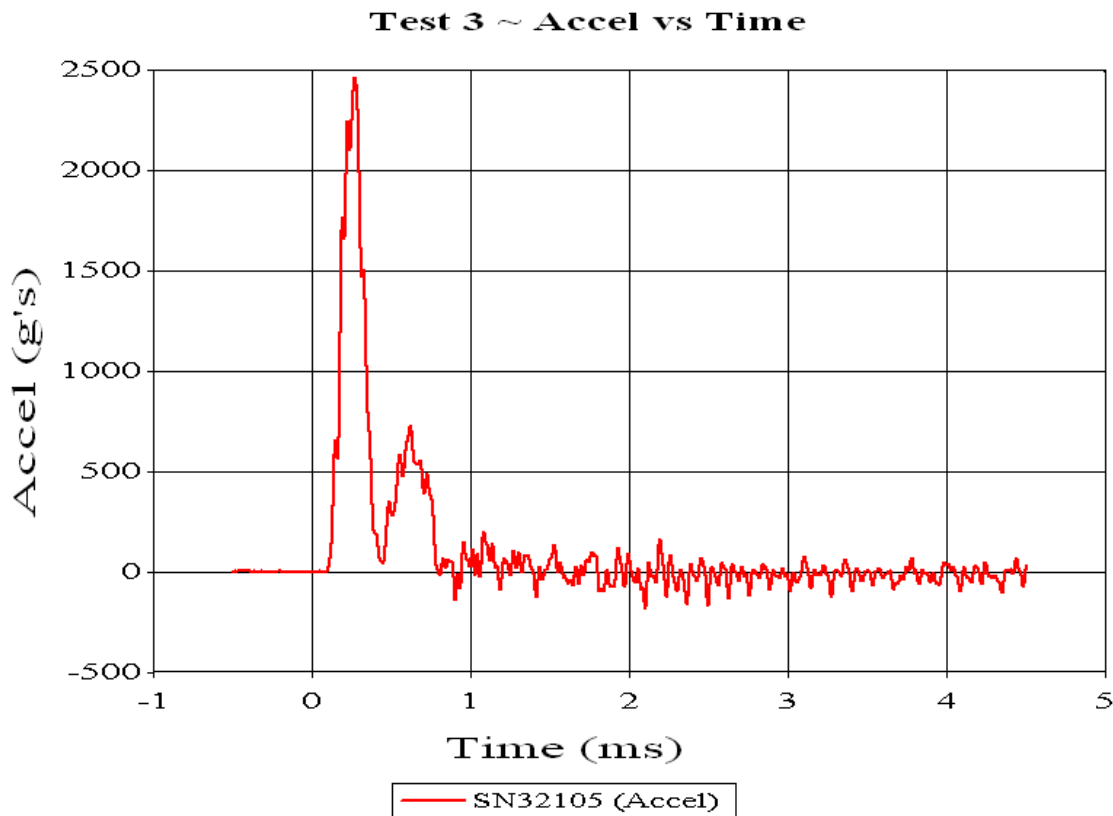
## 2. Testing of Multiple Mitigation Techniques on a Simulated Vehicle Undergoing Blast Loading

*Significant progress has been made testing two different mitigation techniques on a simulated vehicle. By testing a V-shaped hull and polyurea cans individually, as well as together, we can gain a fuller understanding of just how much energy can be mitigated from the blast.*

*Dr. Jarrod Bonsmann and the Dynamic Effects Lab tested two methods of mitigation to reduce peak acceleration experienced by a blast loaded vehicle. To do these tests we used two round plates and kept the distance between them constant. The bottom plate would represent the hull of a vehicle and it was kept at a constant distance above the surface of the saturated sand in which the explosive was buried beneath the center of the plate. The second (top) plate would represent the floor of the passenger compartment and it was connected to the bottom plate by the mitigating structural element. We have run numerous tests with no mitigating mechanism between the two plates so as to be able to measure the effect of the mitigation technique being tried. In the first picture on the left in Figure 10 the two plates are separated by four long slender hollow tubes. When the detonation occurs these four tubes buckle globally, that is a single buckle occurs at the center of the tubes. The buckled tubes can be seen in the photo at the top right. In the picture on the bottom left we have placed four soda cans (which have a large outer diameter and thin walls) between the hull and the passenger compartment. As shown in the photograph on the bottom right of Figure 9, all cans are locally buckled with many buckling sites. That is, they are crushed by the very dynamic load. A typical acceleration signal for the control group is shown in Figure 11.*



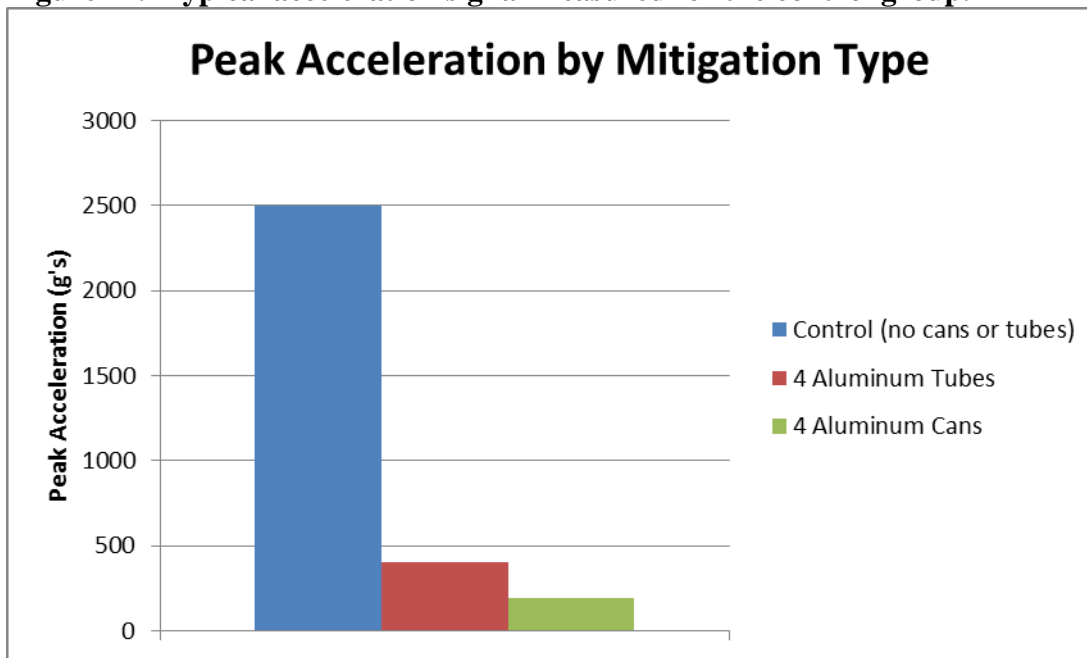
**Figure 10. Pre-test and Post- tests pictures of mitigation by buckling.**



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**Figure 11. Typical acceleration signal measured for the control group.**

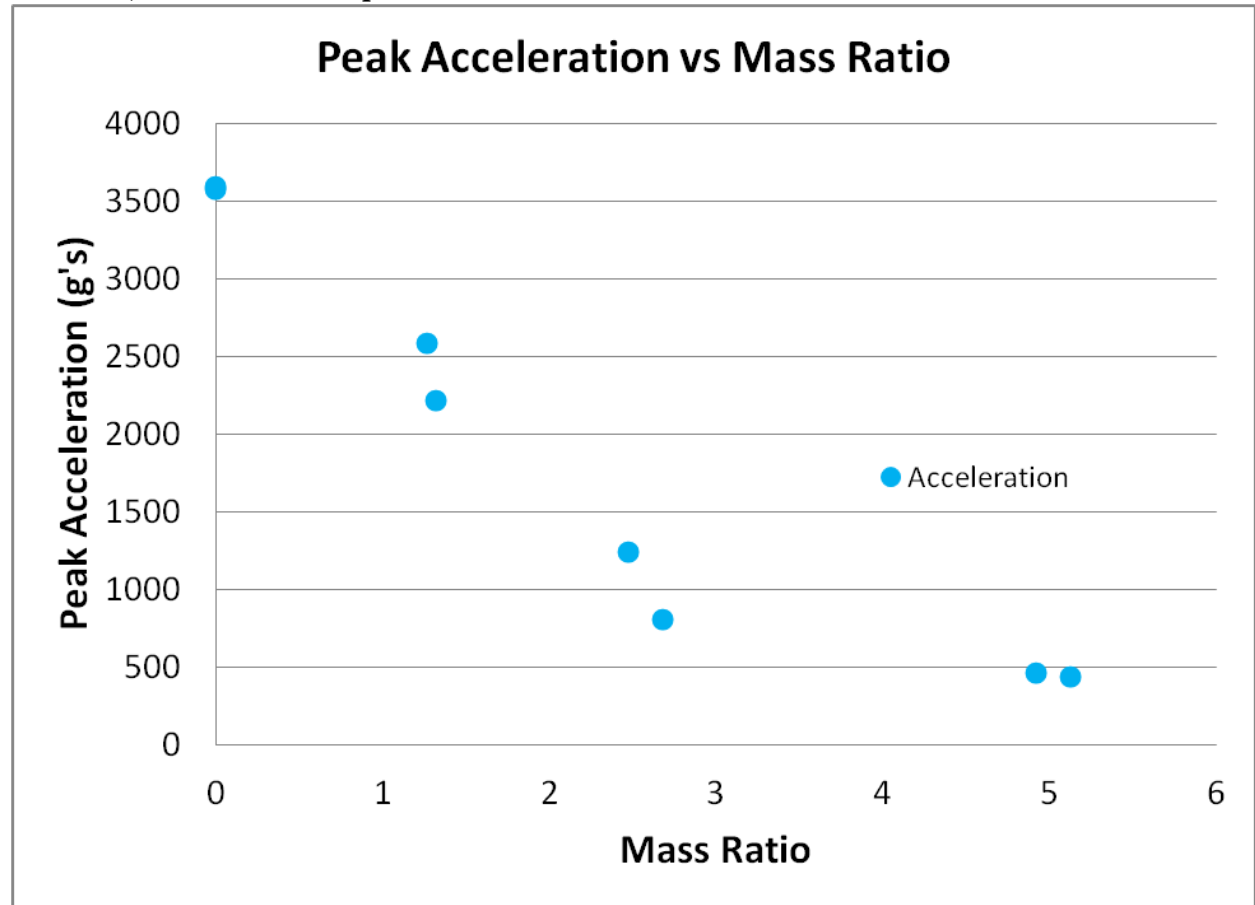


**Figure 12. Peak acceleration of different mitigation methods.**

*Figure 12 shows that aluminum cans are an effective way of mitigation the peak acceleration experienced by a blast loaded vehicle. In regards to Traumatic Brain Injury (TBI), the most important piece of data is the peak acceleration recorded on the plate. The peak acceleration of Figure 12 is the highest value recorded above, or approximately 2500 g's. Since the test is done at small scale. The scaling factor for this particular test is 10, meaning that a person on a full sized vehicle would experience 250 g's.*

*To enhance the effect of using aluminum cans as mitigation, the cans have been coated in polyurea to further reduce the peak acceleration experienced by the vehicle. Figure 13 below shows the effect of*

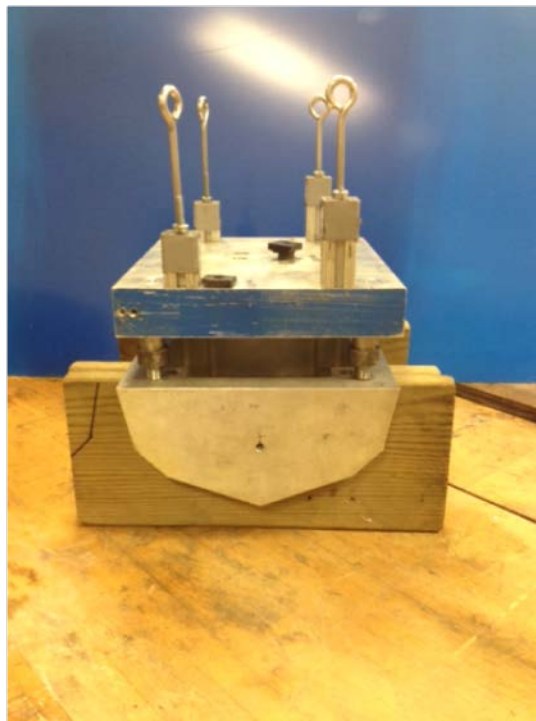
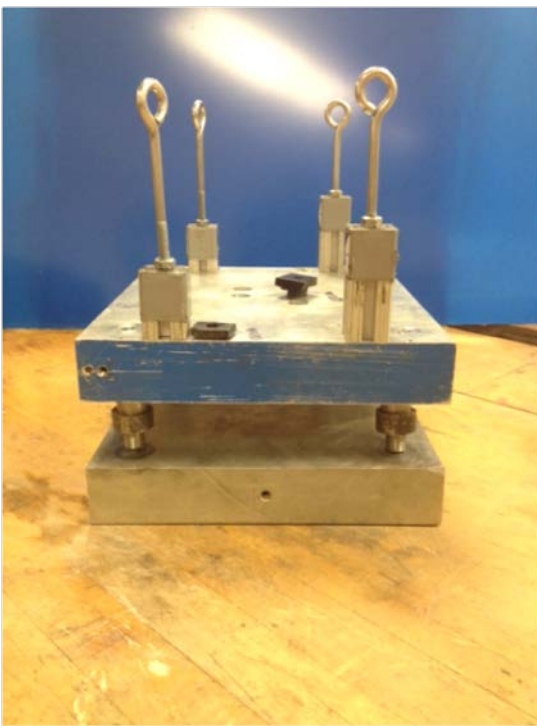
*polyurea coated cans on peak acceleration. Different amounts of polyurea coating, measured in mass ratio, have different reductions in peak acceleration on the blast loaded plate. As the amount of polyurea coating increases, the reduction in peak acceleration increases.*



**Figure 13. Peak acceleration versus can coating mass ratio**

*As can be seen above, the polyurea coated aluminum cans have the lowest peak acceleration of all tests, so they will be used in the current study alongside the V-shaped hull. In our current test matrix, there are four different test plate set ups: flat bottom hull with rigid connectors, V-shaped hull with rigid connectors, flat bottom hull with polyurea coated can connectors, and V-shaped hull with polyurea coated can connectors. Shown in Figures 14 and 15 are the flat bottom and V-shaped bottom with the rigid connectors.*





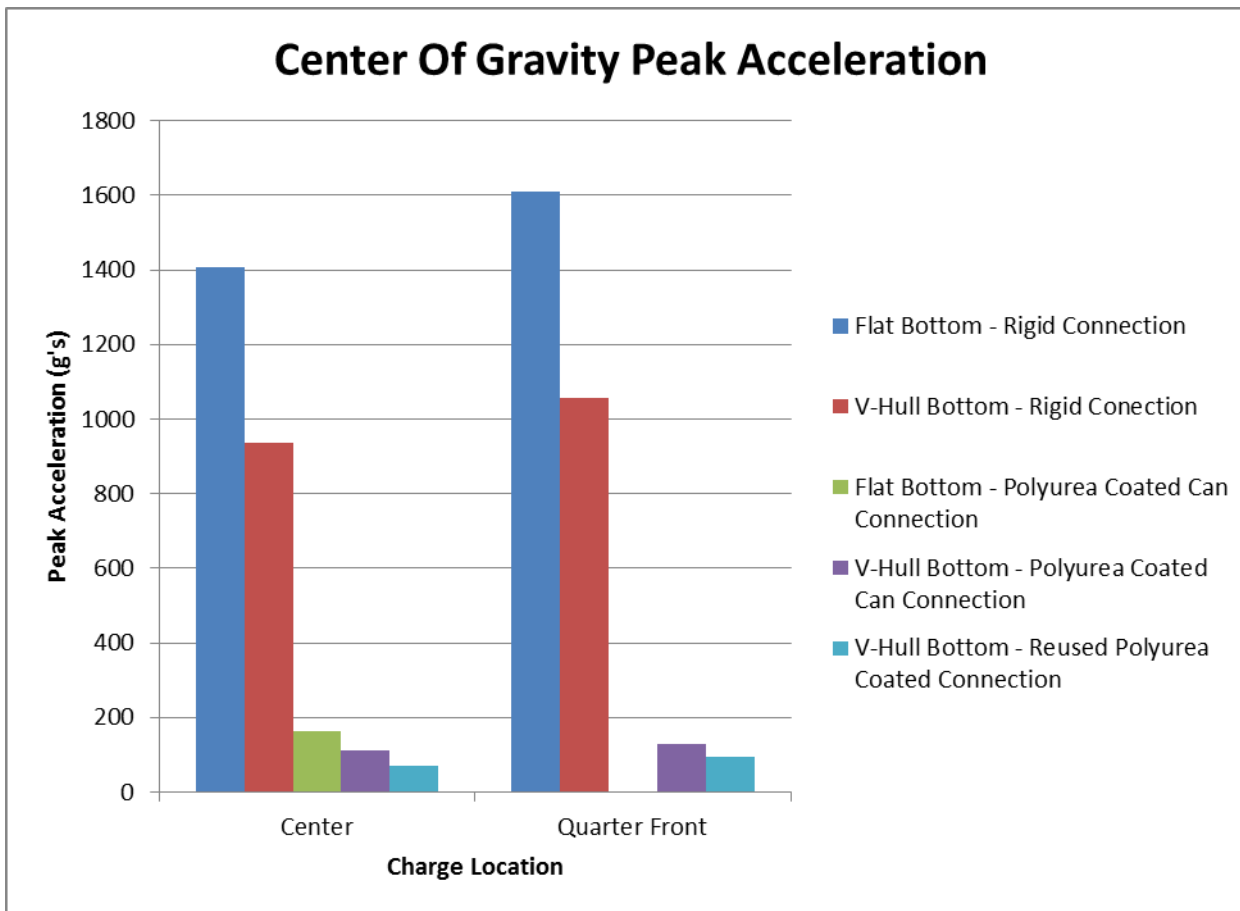
**Figure 14. Flat bottom, rigid connections    Figure 15. V-Hull bottom, rigid connections**

*Each test will be conducted at a constant Stand-off Distance (SOD), meaning that the distance between the bottom of the bottom plate and the top of the sand test bed is held constant. While the Dynamic Effects Lab chooses to use Stand-off Distance as a test parameter, some choose to use the distance to the floor board where the vehicle's passengers would theoretically be sitting. This is a factor that could affect the results and is something that could possibly be explored in future testing.*

*Each of the four different test set-ups will be tested at five different charge locations to see the effects of the mitigation in less idealized settings. The charge will be buried at the center of the plate, a quarter of the plate width to the right of the center, half of the plate width to the right of the center, a quarter of the plate length forward of the center, and half of the plate length forward of the center.*

*To date, all of the center charge tests have been completed, as well as four of the five tests at the quarter front charge location. The results for reduction in peak acceleration are shown in Figure 16 below.*

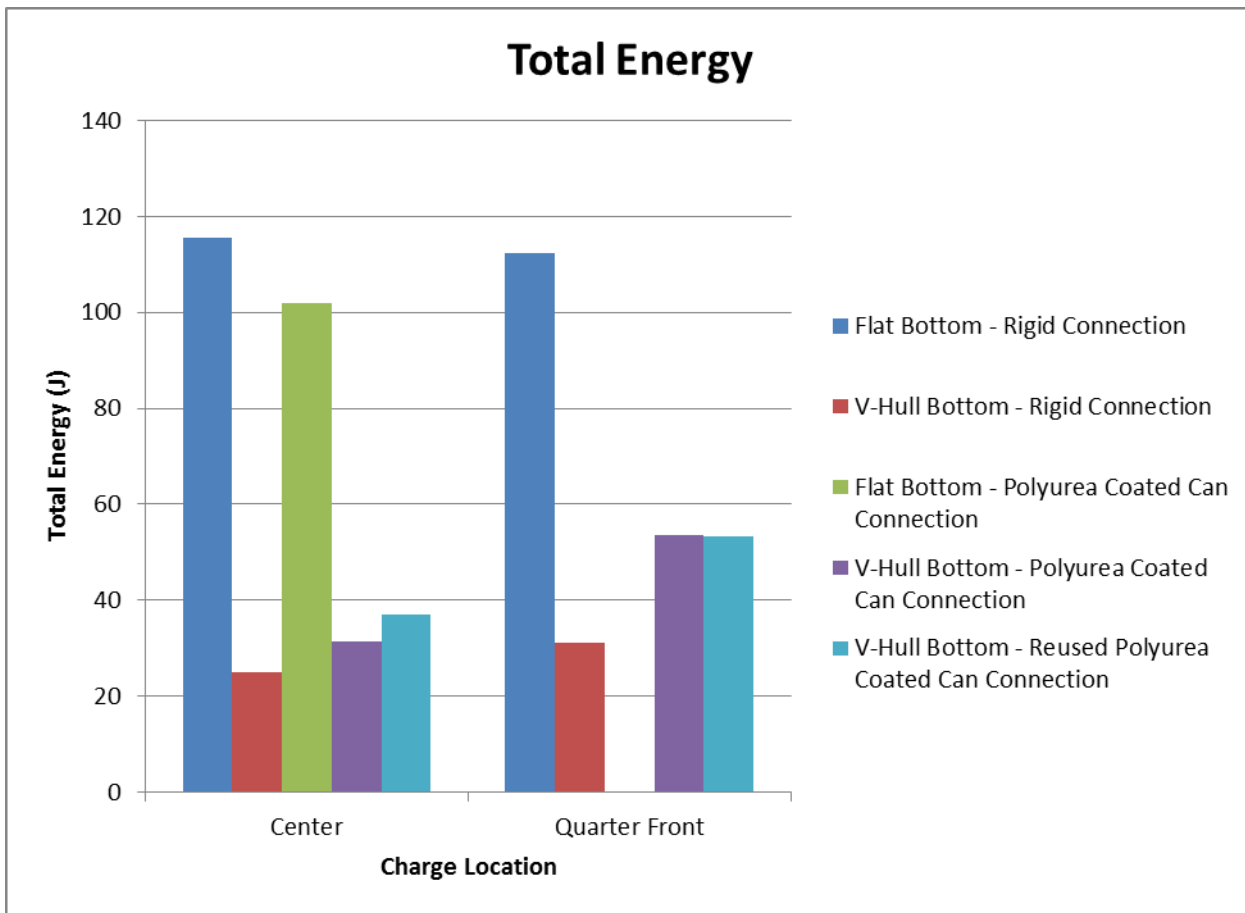




**Figure 16. Peak acceleration by test set-up and charge location.**

*In the center charge location case, the peak acceleration is reduced from 1406 g's in the baseline case to 111 g's when utilizing the V-hull and polyurea coated cans. In the quarter front charge location, the peak acceleration is reduced from 1611 g's in the baseline case to 127 g's when utilizing the V-hull and reused polyurea coated cans. With a scaling factor of 10 on the acceleration signals, both test cases become survivable at large scale when using the v-hull with the polyurea coated cans.*

*Shown below in Figure 17 is the total energy, or the total of linear and rotational kinetic energy, of the system.*



**Figure 17. Total Energy by test set-up and charge location.**

*Future work conducted includes completing the test matrix outlined above by testing at the three remaining charge locations. Once the test matrix is complete, attempts will be made to shorten the height of the polyurea coated cans from 1.5 inches to a shorter distance of around 0.5 inches. Since the scaling factor on these tests is 10, a 1.5 inch polyurea coated can translates to a 15 inch can on a full sized vehicle. Adding such a considerable height to the vehicle has many negative effects, including a higher center of gravity and increased chance of rollover.*

## **KEY RESEARCH ACCOMPLISHMENTS:**

- Quantitative histochemical evidence for damage to brain white matter axon fibers following exposure of rats to underbody blasts resulting in peak vertical accelerations of 100 and 700 Gs, with significant increase in axon injury at 700 compared to 100 Gs.
- Quantitative immunohistochemical evidence for blood brain disruption in the cerebral cortex of rats following exposure to 700 G underbody blasts.
- Quantitative immunohistochemical evidence for a large elevation in vascular levels of von Willebrand Factor 7 days after 100G underbody blasts.
- Quantitative immunohistochemical evidence for an increase in the number of either perivascular or periventricular macrophages or microglia at 24 hr following 700G blast.
- Quantitative molecular biological evidence for significant differences in hippocampal gene expression following exposure to 100 G underbody blasts. A significant reduction in expression of Bcl2 was observed, which could promote brain cell death, and a significant increase in expression of von Willebrand Factor was observed, which is indicative of vascular injury.
- Qualitative histological evidence for axonal injury in several brain regions, including the internal capsule, the corpus callosum, and the cerebellum. Also evidence for astrocyte activation.
- Preliminary evidence for hippocampal neuronal death and cerebellar Purkinje neuronal death at 24 hr after exposure to 700G blast.
- Neurobehavioral evidence for acute and chronic fear and anxiety in conscious rats exposed to 2800G blasts.
- Demonstration that the use of crushable cylinders separating an outer and inner hull can dramatically reduce the G force load on the inner hull. Further demonstrations that application of a poly-urea covering to the cylinders can provide further protection at high G forces and allow for at least partial reinstatement of shock-absorbing cylinder structures. This rebound characteristic could increase the chance of the blast-targeted vehicle to remain in operation.
- Demonstration that the presence of these cylinders results in a load on anesthetized rats of 300G with no mortality at a charge size and standoff distance that result in a load of 2800G and 80% mortality when the cylinders are not present.

## **REPORTABLE OUTCOMES:**

1. Manuscript published in the Journal of Trauma and Acute Care Surgery providing qualitative histologic evidence for brain injury to rats in our underbody blast TBI model. J Trauma Acute Care Surg. 2014 Sep;77(3 Suppl 2) (see Appendix)
2. Results obtained from this project were presented in a poster session at the National Neurotrauma Society meetings in Nashville, TN in August of 2013. The title of the presentation was “Hypobaric Worsens Axonal Injury and Blood Brain Barrier Disruption Induced by Underbody Blast-Induced Hyperacceleration”.
3. Results obtained from this project were also presented in a platform session at the Military Health System Research Symposium held in Ft. Lauderdale, FL in August of 2013. The title of the presentation was “Hypobaric Worsens Axonal Injury and Blood Brain Barrier Disruption Induced by Underbody Blast-Induced Hyperacceleration”.
4. Intellectual property disclosure to the University of Maryland College Park for vehicle hull designs that can mitigate injury caused by underbody blasts.

**CONCLUSION:** At this early stage of this project we can conclude with confidence that underbody blast induced G forces of as little as 100 Gs cause white matter and vascular damage in the brains of rats. We also conclude that in the absence of secondary head impact, ketamine-anesthetized rats survive blast-induced force of at least 2000 Gs but have a high mortality rate after 2800G blasts. We also conclude that even at the G force of 100, significant changes in brain gene expression occur that could affect outcomes. Rats that are conscious during 2800G blasts exhibit acute and chronic fear and anxiety. Finally, our vehicle hull design efforts have been very successful at demonstrating an unexpectedly large reduction in load placed on the floor of a vehicle, using inner and outer hulls separated by crushable cylinders. When anesthetized rats were subjected to a blast force of 2800G in the absence of compressible cylinders, 5 of 6 died. When using the same charge and standoff distance, the presence of the cylinders reduced the G force on the rats to 300G and all animals for 30 days. Application of such a design to military vehicles could dramatically reduce the extent of injuries to the occupants and even allow the vehicle to keep operating, thus increasing the successful completion of the military mission.

## **APPENDICES:**

# Rat model of brain injury caused by under-vehicle blast-induced hyperacceleration

Julie L. Proctor, MS, William L. Fourney, PhD, Ulrich H. Leiste, PhD,  
and Gary Fiskum, PhD, Baltimore, Maryland

**BACKGROUND:** More than 300,000 US war fighters in Operations Iraqi and Enduring Freedom have sustained some form of traumatic brain injury (TBI), caused primarily by exposure to blasts. Many victims are occupants in vehicles that are targets of improvised explosive devices. These underbody blasts expose the occupants to vertical acceleration that can range from several to more than 1,000 G; however, it is unknown if blast-induced acceleration alone, in the absence of exposure to blast waves and in the absence of secondary impacts, can cause even mild TBI.

**METHODS:** We approached this knowledge gap using rats secured to a metal platform that is accelerated vertically at either 20 G or 50 G in response to detonation of a small explosive (pentaerythritol tetranitrate) located at precise underbody standoff distances. All rats survived the blasts and were perfusion fixed for brain histology at 4 hours to 30 days later.

**RESULTS:** Robust silver staining indicative of axonal injury was apparent throughout the internal capsule, corpus callosum, and cerebellum within 24 hours after blast exposure and was sustained for at least 7 days. Astrocyte activation, as measured morphologically with brains immunostained for glial fibrillary acidic protein, was also apparent early after the blast and persisted for at least 30 days.

**CONCLUSION:** Exposure of rats to underbody blast-induced accelerations at either 20 G or 50 G results in histopathologic evidence of diffuse axonal injury and astrocyte activation but no significant neuronal death. The significance of these results is that they demonstrate that blast-induced vertical acceleration alone, in the absence of exposure to significant blast pressures, causes mild TBI. This unique animal model of TBI caused by underbody blasts may therefore be useful in understanding the pathophysiology of blast-induced mild TBI and for testing medical and engineering-based approaches toward mitigation. (*J Trauma Acute Care Surg.* 2014;77:S83–S87. Copyright © 2014 by Lippincott Williams & Wilkins)

**KEY WORDS:** Axonal injury; astrocyte; inflammation; internal capsule; cerebellum.

Approximately 25% of all US combat casualties in Operation Iraqi Freedom and Operation Enduring Freedom have been caused by traumatic brain injury (TBI), with most of these injuries caused by explosive munitions such as bombs, land mines, improvised explosive devices (IEDs), and missiles.<sup>1,2</sup> Little is known regarding the pathophysiology of “blast TBI.” The majority of animal research on blast TBI has focused on one aspect of these explosions, the blast overpressure.<sup>3–6</sup> Most of these studies use a model in which a gas-driven pressure wave was delivered via a long shock tube, directly to the immobilized animal’s head or body. A multitude of physical forces may play a role in blast TBI, including blast overpressure, thermal and chemical components, shockwave, and hyperacceleration of the brain. We hypothesize that this extreme hyperacceleration, with subsequent rapid deceleration, is responsible for many aspects of blast TBI.

Acceleration may be particularly important for the large number of soldiers and others injured while being occupants of armored vehicles targeted by IEDs. Such explosions result in a very short but very intense acceleration of the vehicle and its occupants. The Dynamic Effects Laboratory at the University of Maryland School of Engineering has used small-scale testing to evaluate the loads applied to personnel carriers when a buried explosive detonates beneath them.<sup>7,8</sup> Adaptation and scaling of this model to allow animal injury in a similar explosive environment could provide a completely new, clinically relevant model of blast TBI that encompasses many of the physical forces including the extreme hyperacceleration. As a first step toward this goal, this study tested the hypothesis that relatively low underbody blast-induced accelerations of 20 G and 50 G result in histologic evidence for mild TBI in the absence of obvious injury to other vital organs.

## MATERIALS AND METHODS

### Underbody Blast-Induced Hyperacceleration

The device used to induce underbody blast-induced acceleration consists of an aluminum water tank 3 ft long × 2 ft wide × 2 ft deep in which a platform is located that supports two thick aluminum plates, each 15 in square and 1.5 in thick (Fig. 1). The two plates are separated by a styrofoam pad of the same dimensions, which absorbs some of the force transmitted between the plates. The plates and pad travel vertically in response to a blast in the water tank, guided by poles located in

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This work was presented at the 2013 Military Health System Research Symposium, August 12–15, 2013, in Fort Lauderdale, Florida.

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holes in each corner of the plates and pad. The two cylinders secured to the top of the plate each house an anesthetized rat that is wrapped in a thick cotton “blanket” to minimize secondary movement within the cylinders. An explosive charge of 0.75-g pentaerythritol tetranitrate is placed in the water precisely under the center of the plate at distances that generate precise, maximal G forces in these experiments of between 20 G and 50 G. Standoff distances were determined previously at the Dynamics Effects Laboratory and measured during the animal experiments, using accelerometers. When detonated, the explosion causes the plate containing the two rats to accelerate upward extremely rapidly to heights of less than 4 in, followed by a return down to the original location. Pressure sensors located immediately next to the rat heads indicated that they were exposed to less than 1-psi increase in pressure following the explosion. Figure 1 provides examples of the accelerometer measurements performed during these tests, demonstrating reproducibility between two different blasts at both 20 G and 40 G and differences observed at 20, 40, and 60 G, with peak accelerations occurring at 5, 4, and 3 milliseconds, respectively.

## Animal Experiments

All animal procedures were performed in accordance with the University of Maryland School of Medicine Institutional Animal Care and Use Committee, the US Army Animal Care and Use Review Office, and the US Air Force Animal Use Program Office of Research Oversight and Compliance. At approximately 10 minutes before each blast, two adult male Sprague-Dawley rats (300–350 g) were deeply anesthetized by intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg). Immediately following the underbody blast, the rats were removed from the cylinders, and their respiratory rates were compared with those recorded before the blast. No changes in respiration were observed. All animals were fully conscious within 90 minutes after the blast and appeared unharmed. In addition to the 25 anesthetized rats subjected to blast-induced hyperacceleration, 10 sham rats were anesthetized but not used in blast experiments.

## Tissue Preparation

At different times following the blasts or sham anesthetization, rats were heavily reanesthetized by intraperitoneal

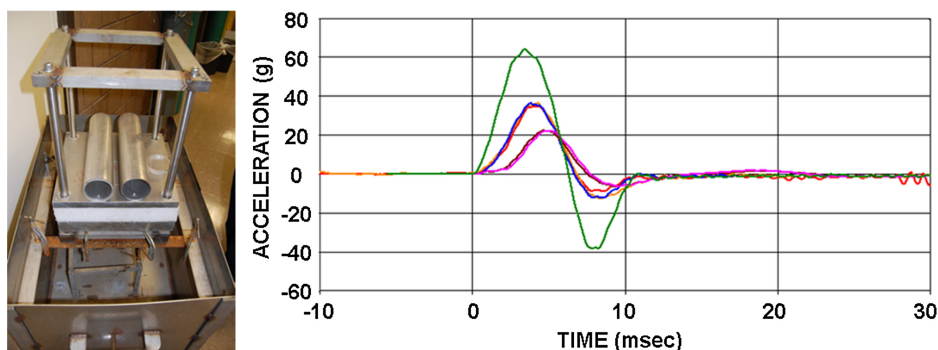
injection of ketamine (160 mg/kg) and xylazine (20 mg/kg) and transcardially perfused with 4% paraformaldehyde plus 2.5% acrolein.<sup>9</sup> Brains were removed from the skull and transferred into 30% sucrose. Once brains sunk to the bottom of the container, they were cut (40  $\mu$ m) on a freezing sliding microtome, yielding 12 series per animal, and were kept in cryoprotectant ( $-20^{\circ}\text{C}$ ) until further processing was initiated.

## Pathology

None of the 25 rats used in the blast experiments exhibited any evidence of injury to the lungs, heart, liver, or spleen upon inspection following thoracotomy during perfusion fixation.

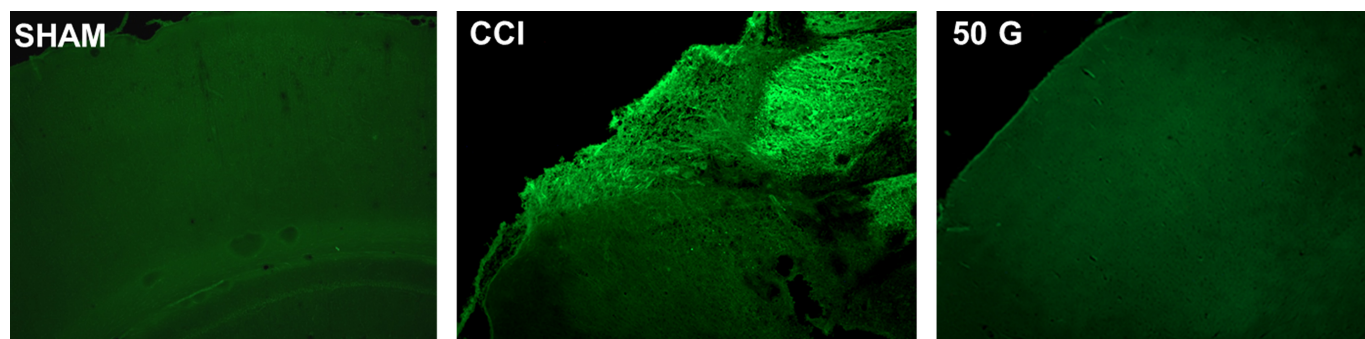
Staining of brain sections with Fluoro-Jade B (FJB) was used to detect dead or dying neurons.<sup>9,10</sup> Free floating brain sections were rinsed free of cryoprotectant with KPBS, mounted on Vectabond-treated PLUS superfrost glass slides and dried overnight at  $50^{\circ}\text{C}$ . Slides were sequentially dipped in the following: 100% EtOH (3 minutes), 70% EtOH (1 minute), deionized  $\text{H}_2\text{O}$  (1 minute), 0.06%  $\text{KMnO}_4$  (15 minutes),  $\text{H}_2\text{O}$  (2 minutes), and 0.0005% solution of FJB (30 minutes). Slides were then dipped in  $\text{H}_2\text{O}$  four times to five times and were allowed to dry for 30 minutes at  $50^{\circ}\text{C}$  before being cleared in xylene and coverslipped with DPX mounting media.

The amino cupric silver method of de Olmos was used to stain free-floating 40- $\mu$ m tissue for the identification of damaged and degenerating axons. Our staining procedure closely followed the detailed protocol described by Tenkova and Goldberg.<sup>11</sup> Before staining, all glassware was cleaned in 50% nitric acid. Sections were rinsed free from cryoprotectant and incubated in 4% paraformaldehyde ( $4^{\circ}\text{C}$ ) for 1 week before staining to block nonspecific labeling of neurons. Sections were then rinsed with deionized  $\text{H}_2\text{O}$  and incubated in preimpregnation buffer (cupric silver) for 1 hour at  $50^{\circ}\text{C}$  then at room temperature overnight. The next day, sections were exposed to the following solutions at room temperature: 100% acetone (30 seconds), impregnation buffer (silver diamine) solution for 35 minutes, reduction agent (formaldehyde with citric acid) for 2 minutes, bleaching solution (potassium ferricyanide) for 20 minutes, deionized  $\text{H}_2\text{O}$  for 3 minutes, and stabilization buffer (thiosulfate solution) for 10 minutes. All solutions were made fresh immediately before use, and sections were carefully shielded from direct light during all staining procedures. After staining, sections were mounted in



**Figure 1.** Device used for underbody blast-induced hyperacceleration experiments and accelerometer recordings obtained during the blasts. Tracings show highly reproducible accelerations generated by duplicate blasts at both 20 G and 40 G as well as a single blast at 60 G.





**Figure 2.** FJB staining for dead or dying cortical neurons after exposure of rats to head impact or underbody blast. Sham animal was anesthetized with ketamine and perfusion fixed 7 days later. One rat was used in a CCI model of moderate TBI and perfused 7 days later. One ketamine-anesthetized rat was subjected to a 50-G underbody blast and perfused 7 days later.

50% ethanol onto subbed PLUS slides, dehydrated with ethanol and xylene, and subsequently coverslipped with DPX mounting media.

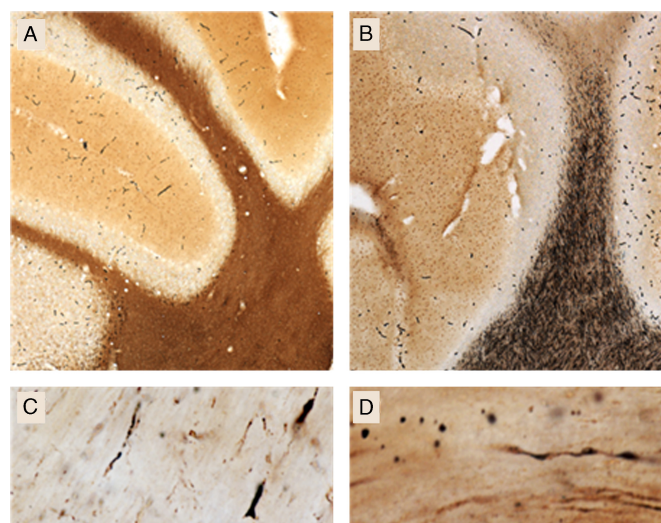
### Immunohistochemistry

Free-floating sections were single labeled with antibodies against the astrocyte marker, glial fibrillary acidic protein (GFAP),<sup>9</sup> by rinsing multiple times in 0.05-M KPBS buffer before and after exposure to the following: 1% solution of sodium borohydride for 20 minutes; primary antibody diluted in 0.05-M KPBS + 0.4% Triton-X for 48 hours (Dako Anti-GFAP; 1:150K); biotinylated secondary antibody diluted in 0.05-M KPBS + 0.4% Triton-X 1:600 for 1 hour; and incubation in Vectastain A/B solution (1:222) for 1 hour. Tissue was then rinsed before and after 12-minute incubation in Ni-DAB solution with 0.175-M sodium acetate buffer. After a final rinse in KPBS buffer, slices were mounted on slides, dehydrated, and coverslipped with DPX mounting media. The sections were examined with Nikon Eclipse E800 microscope and captured using StereoInvestigator software.

## RESULTS

Neuronal death is typically observed within 1 week of injury in most rodent TBI models. Neuronal death or degeneration that occurs in this period is often detected histologically, using a FJB stain, which selectively labels dead or dying neurons.<sup>9,10</sup> Figure 2 compares FJB staining present in the frontal cerebral cortex of a sham rat, perfusion fixed 7 days after ketamine anesthesia, to that observed 7 days following moderate injury induced using a controlled cortical impact (CCI) model, which uses a pneumatic device to directly impact the cortical surface.<sup>12</sup> Representative tissue from the CCI model was obtained from our rodent brain bank and not generated as part of this study. Extensive staining was apparent in the brain from the rat that underwent the CCI, whereas virtually no staining was detectable in the sham rat. In contrast to the staining exhibited by the positive control animal that was previously subjected to cortical impact, no FJB staining was observed at 7 days in a rat subjected to a 50-G underbody blast. Examination of 12 coronal sections representing the entire brain detected no FJB-stained neurons in 23 rats used in the 50-G underbody blast experiments or in 2 rats used in 20-G underbody blast experiments.

Diffuse axonal injury is often observed in rodent brain injury models and can be detected by staining of axons with silver-containing reagents.<sup>10,13</sup> With the use of the de Olmos silver staining method, widespread staining was evident at 7 days after 50-G blasts. Staining was most striking in the internal capsule, corpus callosum, and cerebellum (Fig. 3). Abnormal axon morphologic finding was represented by undulations and bulb-like swellings (Fig. 3, bottom panels). Additional axonal staining was observed in tracts serving the thalamus, while very few fibers in the olfactory bulb or anterior commissures showed evidence of axonopathy. Where silver staining was observed in superficial layers of the brain, it occurred more commonly in the ventral rather than dorsal cortical regions. Relatively little axonal silver staining was observed in noninjured sham animals.



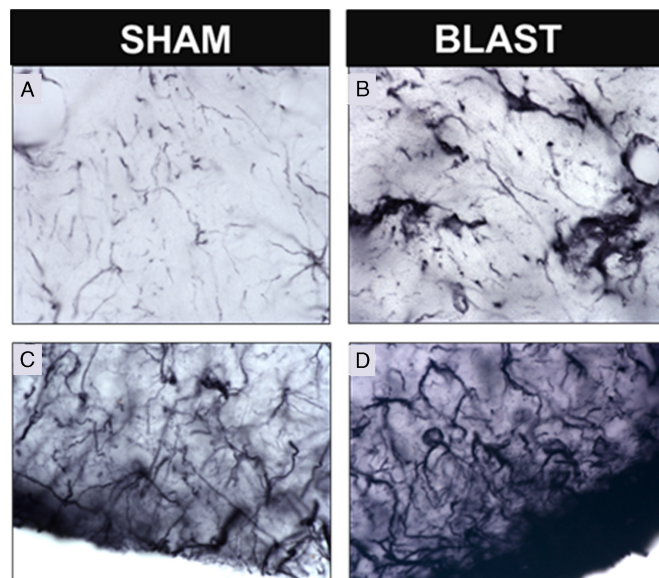
**Figure 3.** de Olmos silver staining of rat brain 7 days following a 50-G underbody blast. Widespread staining demonstrates axonopathy in white matter structures of the cerebellum in rats exposed to underbody blast (B) as compared with sham controls (A). High magnification confirms the presence of abnormal swellings and varicosities present in silver-stained axons within the internal capsule (C and D) following blast exposure.

Axonal silver staining was detectable in the internal capsule of animals subjected to 20-G underbody blasts and perfusion fixed at either 24 hours or 7 days. Evidence for even earlier axonal injury was obtained with brains fixed 3 hours after 50-G blasts (Fig. 4). In contrast, silver staining was much less obvious at 30 days after blast than at 3 hours or 7 days (Fig. 4).

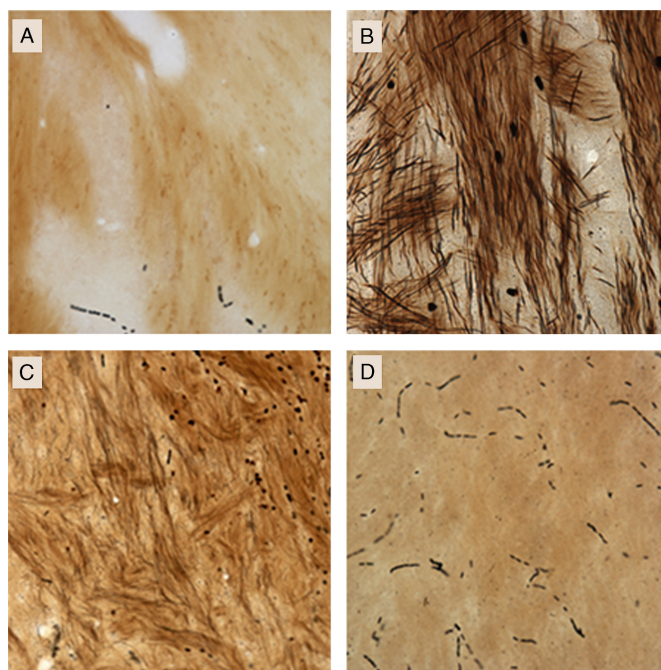
Cellular inflammatory responses are another hallmark of TBI. These responses are characterized by proliferation, migration, and morphologic transformation of astrocytes and microglia, which constitute approximately 50% of the mass of the human brain.<sup>14</sup> Surprisingly, we obtained no convincing evidence for microglial activation following 20-G or 50-G blasts at any outcome times (not shown). Nevertheless, we consistently observed astrocyte accumulation near ventral cortical surfaces, hypothalamic regions, and the internal capsule (Fig. 5). In addition, many astrocytes present in different brain regions exhibited large cell bodies, indicative of activation. Unlike the silver staining of axons, which declined between 7 days and 30 days after the blast, these cellular inflammatory reactions persisted and possibly increased during this period.

## DISCUSSION

To the best of our knowledge, these experiments represent the first to test for the effects of specifically underbody blast-induced hyperacceleration on the brains of laboratory animals. The blast paradigm was designed to test for the effects of this form of acceleration on the brains of rats in the absence of



**Figure 5.** Astrocyte activation near hypothalamic regions at 7 days after a 50-G underbody blast. Intensities of GFAP-stained astrocytes were greater in animals exposed to underbody blasts compared with sham-treated rats. Astrocyte activation was observed in the internal capsule (B) and regions of the hypothalamus (D) as compared with these regions in shams (A and C).



**Figure 4.** de Olmos silver staining of rat brain internal capsule at early and late time points following 50-G generated blasts. Intense internal capsule staining is induced with 50-G forces (B–D), which appears to dissipate with time as compared with ketamine-anesthetized sham controls (A). A, Sham; B, 50 G at 3 hours after blast; C, 50 G at 7 days after blast; D, 50 G at 30 days after blast.

exposure to any significant blast overpressure. Several conclusions can be made. (1) Adult rats can be subjected to blast-induced hyperacceleration at maximal G forces of at least 50 G with a 100% survival rate, provided they are protected against secondary impact injuries. (2) FJB-detectable neuronal death does not occur following exposure of rats to either 20-G or 50-G underbody blasts. (3) Diffuse axonal injury occurs in several brain regions, as early as 4 hours after 20-G or 50-G underbody blasts and is still evident at 7 days, based on silver staining of axonal fibers. (4) Despite the lack of evidence for substantial microglial activation, astrocyte activation occurs within 7 days and persists for 30 days following 20-G or 50-G underbody blasts. (5) Based on these qualitative histologic findings, we conclude that rats subjected to 50-G blasts and possibly even 20-G blasts experience mild TBI.

This unique initial approach to understanding the effects of underbody blasts on the brain has several limitations. (1) We specifically designed the device for exposing rats to underbody blasts to test specifically for the effects of acceleration, without secondary effects of potential head impact. Clearly, many victims of TBI who are occupants of vehicles targeted by IEDs detonated underneath the vehicles are subjected to both acceleration, head impact, and other injuries. Therefore, polytrauma animal models may be more clinically relevant.<sup>5,15</sup> (2) Only two maximal G forces were used, which are much lower than maximum survivable G forces experienced by occupants within targeted vehicles. We purposely used relatively low loads in this initial study to avoid mortality and injury to other vital organs. Studies are in progress exposing rats to underbody blasts at much higher G forces in the range of 100 G to 2,000 G. Recently, one study published evidence of head acceleration exceeding 3,000 G in a



blast tube, porcine model of survivable free field blast exposure.<sup>16</sup> (3) Neuronal death was only measured using one method, that is, FJB tissue staining. It is therefore possible that other methods, such as active caspase 3 immunostaining, could detect neuronal death not detected by FJB. (4) Diffuse axonal injury was only evaluated using the de Olmos silver staining method. Although this approach is used extensively, additional procedures, for example, immunohistochemical detection of phosphorylated tau protein and beta-amyloid precursor protein, could provide helpful validation.<sup>17,18</sup> (5) The results obtained from the silver staining of degenerative neurons and the GFAP immunostaining of astrocytes demonstrating activated morphology are at this juncture purely qualitative. Studies are in progress to obtain quantitative results for these and other histologic outcome measures. (6) No quantitative assessment of behavior was performed. Now that we have obtained histologic evidence for mild TBI following low-G underbody blasts, we are developing neurobehavioral tests sensitive enough to detect neurologic alterations at these and higher G forces.

#### AUTHORSHIP

J.L.P. performed all of the histology and generated all of the results presented in the article. W.L.F. and U.H.L. designed and built the device used for the underbody blast TBI model, were present at every experiment, and conducted the blasts and performed the accelerometer measurements. G.F. is the principal investigator of the grants that supported the work and conceived the study design. He was also present at every experiment. All authors contributed to the writing of this manuscript.

#### DISCLOSURE

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